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BIOLOGY OF PROGESTERONE ACTION DURING PREGNANCY RECOGNITION AND MAINTENANCE OF PREGNANCY

Thomas E. Spencer and Fuller W. Bazer

Center for Animal Biotechnology and Genomics and Department of Animal Science, Texas A&M University, College Station, Texas 77843-2471

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1. ABSTRACT

Progesterone is the hormone of pregnancy and unequivocally required in all mammals for maternal support of conceptus (embryo/fetus and associated membranes) survival and development. The actions of progesterone are mediated by the progesterone receptor (PR). However, the endometrial lumenal (LE) and glandular epithelia (GE) of a number of species exhibit a loss of PR expression prior to the stages of uterine receptivity and implantation. In sheep, PR expression becomes undetectable in the endometrial LE after Day 11 and then in the GE after Day 13. Loss of PR in the GE appears to be required for onset of differentiated functions in terms of production of secretory proteins, such as uterine milk proteins (UTMP) and osteopontin (OPN). Therefore,

the actions of progesterone on endometrial epithelia during most of gestation appear to be mediated by the endometrial stroma that remains PR-positive throughout pregnancy. Stromal cells produce several growth factors, such as hepatocyte growth factor (HGF) and fibroblast growth factors-7 and –10 (FGF-7, FGF-10), that have receptors expressed specifically in the endometrial epithelia. These factors may be progesterone-responsive and mediate epithelial-mesenchymal interactions that are crucial for support of pregnancy. Studies of the uterine gland knockout (UGKO) ewe indicate that uterine glands and, by default, their secretions are required for peri-implantation conceptus survival and growth. A complex servomechanism, involving hormones from the ovary and conceptus as well as endogenous betaretroviruses expressed in the endometrial LE and GE, is proposed to regulate endometrial gland differentiation and function during gestation. At estrus, estrogen increases PR expression in the endometrial epithelia. High levels of endogenous Jaagsiekte sheep retroviruses (enJSRVs) are expressed in the PR-positive endometrial LE and GE in response to increasing progesterone and are hypothesized to stimulate trophoblast proliferation and production of interferon (IFN) tau. IFN tau, the pregnancy recognition hormone produced by the trophoblast from Days 10 to 21, acts in a paracrine manner on the PR-negative endometrial LE and superficial GE to inhibit transcription of estrogen receptor alpha (ER) and oxytocin receptor (OTR) genes. These actions of IFN tau maintain progesterone production from the corpus luteum by abrogating release of luteolytic pulses of prostaglandin F2 alpha (PGF) from the endometrial epithelium. The antiluteolytic effects of IFN tau are dependent on progesterone. Progesterone stimulation over 8-10 days suppresses expression of the PR gene in the LE and then GE. Loss of the PR in the LE is concomitant with decreases in mucin glycoprotein one (MUC-1), an inhibitor of blastocyst implantation. As the conceptus begins implantation on Day 15, the binucleate trophectodermal cells then differentiate and produce placental lactogen (PL), a member of the prolactin (PRL) and growth hormone (GH) family. PL stimulates GE proliferation and production of secretory proteins, such as UTMP and OPN. Interestingly, the effects of PL on the GE appear to require the absence of PR and prior exposure to IFN tau. During mid-pregnancy, the mononuclear trophectodermal cells produce GH that can also act on a progestinized uterus to stimulate GE hypertrophy and secretory function. The actions of this servomechanism are proposed to stimulate GE hyperplasia from Days 20 to 50 and then GE hypertrophy and maximal differentiated function after Day 50 when the majority of fetal growth and development occurs during gestation.

2. PREGNANCY RECOGNITION SIGNALING IN SHEEP

Ruminant species are spontaneous, seasonal ovulators that undergo uterine-dependent estrous cycles until establishment of pregnancy (see 1-3). The estrous cycle is dependent on the uterus as it is the source of the luteolysin, prostaglandin F2 alpha (PGF). During the estrous cycle, cell-specific changes in the expression of hormone receptors control development of the endometrial luteolytic mechanism. Maternal recognition of pregnancy in sheep requires that the conceptus (embryo/fetus and its associated membranes) elongate from a blastocyst to tubular and then filamentous form to produce IFN tau, which prevents development of the endometrial luteolytic mechanism. This antiluteolytic effect of IFN tau results in the maintenance of a functional corpus luteum (CL) and, hence, secretion of progesterone. Progesterone, the hormone of pregnancy, is essential to create a uterine environment that supports events critical to successful development of the conceptus to term.

2.1. Expression of receptors for estrogen (ER) and progesterone (PR)

Alterations in tissue- and cell-specific expression of uterine ER alpha and PR during the estrous cycle and early pregnancy cannot be attributed solely to changes in circulating steroid hormone concentrations. Thus, locally produced, paracrine-acting factors are likely involved in regulating uterine ER and PR gene expression. Undoubtedly, tissue- and cell-type specific changes in ER and PR expression are involved in epithelial-mesenchymal interactions which regulate uterine physiology, including development of the luteolytic mechanism, uterine growth and development, and myometrial quiescence based on results from studies of sheep (4), cattle (5), pig (6), western spotted skunk (7), rhesus monkey (8), and human (9).

2.1.1. Sheep

Mechanisms regulating responses of the ovine uterus to endocrine and paracrine signals during the estrous cycle and pregnancy entail tissue- and cell-specific regulation of ER and PR expression (4,10). In cyclic ewes, ER mRNA and protein expression in total endometrium is highest on Day 1, declines between Days 1 and 6, and then increases between Days 11 and 15. However, in pregnant ewes, endometrial ER mRNA and protein expression in total endometrium remains low between Days 11 and 15 and increases only slightly between Days 15 and 25. Expression of PR mRNA and protein in total endometrium of cyclic ewes is highest on Day 1, decreases between Days 1 and 11, and then increases between Days 13 and 15. In pregnant ewes, PR mRNA and protein expression in total endometrium is low on Day 11, increases between Days 11 and 17, and decreases again between Days 17 and 25.

Studies in a number of mammalian uteri discovered cell type-specific changes in hormone receptor expression during the estrous/menstrual cycle and pregnancy. Similarly, temporal and spatial alterations in expression of ER and PR genes occur during the estrous cycle and early pregnancy in the endometrial epithelia and stroma that were not revealed in analyses of total endometrium (4,10). During the estrous cycle, the endometrium releases luteolytic pulses of PGF that induce regression of the CL (luteolysis). The source of luteolytic PGF pulses is the endometrial LE and superficial GE (sGE), because they express OTR and are the only cell types that expresses cyclooxygenase 2 (COX-2), an important enzyme in PG synthesis (11,12). The endometrial luteolytic mechanism that develops in the LE

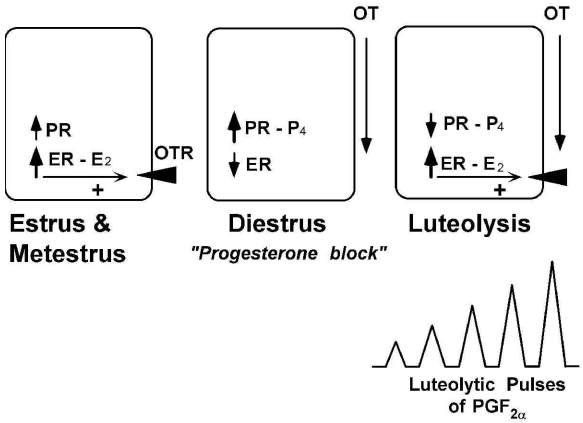

Figure 1. Schematic illustrating regulation of hormone receptor expression in the ovine uterine endometrial epithelium during the estrous cycle. During estrus and metestrus, OTR are present on the uterine epithelia, because estrogen levels are high and increase expression of ER and OTR. The PR is present, but low circulating levels of progesterone result in insufficient numbers of activated PR to suppress ER and OTR synthesis. During diestrus, endometrial ER and estradiol in plasma are low, and progesterone levels begin to increase with formation of the CL. Progesterone acts through its receptor to maintain the "progesterone block" to ER and OTR synthesis for 8 to 10 days. During late diestrus (~Days 11 to 12 of the cycle), progesterone negatively autoregulates PR gene expression, which allows for increases in ER and OTR synthesis. The increase in OTR expression is facilitated by increasing secretion of estrogen by ovarian follicles and then decreasing secretion of progesterone. The pulsatile release of oxytocin from the CL and posterior pituitary activates OTR on the endometrial epithelium and induces release of luteolytic pulses of PGF to regress the CL. Legend: E2, estrogen; ER, estrogen receptor alpha; OT, oxytocin; OTR, oxytocin receptor; P4, progesterone; PGF, prostaglandin F2 alpha; PR, progesterone receptor. Adapted from (Bazer 1992).

and sGE requires sequential effects of progesterone, estradiol-17beta and oxytocin, acting through their respective receptors (figure 1). At estrus (Day 0), estrogen levels peak from an ovulatory Graafian follicle and increase ER, PR and OTR expression. Progesterone from the newly formed CL stimulates accumulation of phospholipids that serve as substrate for phospholipase A2 to liberate arachidonic acid for PGF synthesis and secretion. During diestrus, progesterone levels increase and act via PR to "block" expression of ER and OTR in the LE and sGE (13). Therefore, ER and OTR expression is not detected on Days 5 to 11 of the cycle. The precise molecular mechanism whereby progesterone suppresses ER gene transcription is unknown. However, the effects of progesterone on OTR gene expression may be indirect through suppression of ER, because the rat OTR gene contains ER response elements that mediate estrogen effects (14). Continuous exposure of the uterus to progesterone for 8 to 10 days down-regulates expression of PR in LE after Day 11 and GE after Day 13 (15), allowing for rapid increases in expression of ER on Day 13 and then OTR on Day 14 (16,17). The mechanism whereby progesterone negatively autoregulates expression of PR is not known, but may involve PR-mediated decreases in PR gene transcription (18,19). Oxytocin, secreted beginning on Day 9 from the posterior pituitary and/or CL, then induces release of luteolytic pulses of PGF from LE and sGE on Days 14 to 16 (11). In response to 4-5 luteolytic pulses of PGF over a 25 h period, the CL then undergoes functional and structural regression, allowing for the ewe to return to estrus and complete the 17-day cycle.

Clearly, temporal and spatial changes in expression of ER and PR are critical to changes in uterine biology during both the estrous cycle (luteolysis) and establishment and maintenance of pregnancy. During pregnancy, both ER and PR gene expression in LE and GE are either absent or below detectable limits between Days 15 and 25. Between Days 20 and 140 of gestation, ER and PR are absent from LE and GE, but PR remains abundant in stroma and myometrium (T.E. Spencer, unpublished results). Interestingly, the remodeling and morphogenesis of the endometrial GE that occurs during pregnancy may require the absence of PR expression (4,20,21). In pregnant ewes, PR become absent in uterine epithelia when circulating concentrations of progesterone are high. In the neonatal ewe, PR are abundant in uterine epithelia, but circulating concentrations of progesterone are below detection (22). In the mouse uterine epithelium, progesterone inhibits estrogen-induced cyclin D1 and cyclin-dependent kinase 4 (cdk4) nuclear translocation, cyclin E- and cyclin A-cdk2 kinase activation, and cell proliferation (23). Therefore, liganded PR is likely to inhibit epithelial morphogenesis due to negative effects on progression through the cell cycle. Therefore, the absence of the PR after Day 15 in the GE may be important, because the endometrial glands undergo a pregnancy-dependent program of hyperplasia from Days 16 to 50 and then hypertrophy from Days 50 to term.

2.1.2. Mice

In mice, differential uterine expression of ER alpha and PR also correlate with uterine preparation for implantation and decidualization (24). In the peri-implantation mouse uterus, ER is expressed in LE and GE on Days 1 and 2 of pregnancy, while on Days 3 and 4 of pregnancy ER is localized primarily in stromal cells in addition to its presence in the epithelium. Following implantation on Day 5, ER is higher in LE and GE, but declines in stroma at implantion sites. On Days 6-8 of pregnancy, ER is primarily localized to the secondary decidual zone with more intense localization in subepithelial cells at the mesometrial pole. ER is low to undetectable in the primary decidual zone and implanting conceptuses. The expression pattern for PR is also dynamic in the peri-implantation mouse uterus. On Day 1, PR is low to undetectable, increases in LE and GE on Day 2, and is expressed in both epithelia and stroma on Days 3 and 4. By Day 5, PR expression is restricted to the stroma with increased abundance at implantation sites. On Days 6 to 8, PR increases throughout the decidual tissue. This compartmentalized expression of ER and PR suggests cell specific and temporal changes in the sites of action of both

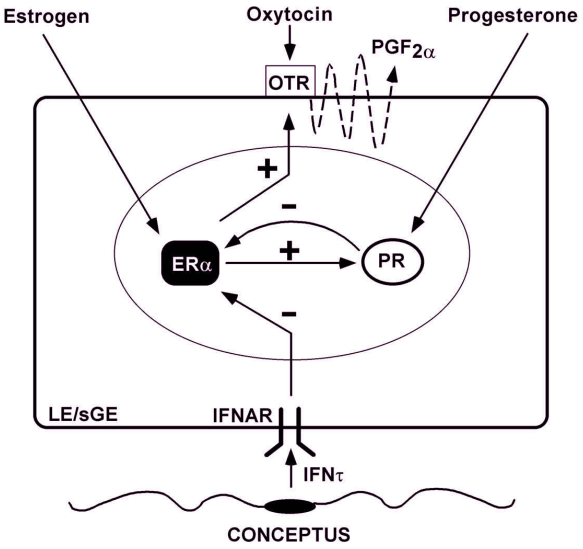

Figure 2. Schematic illustrating current working hypothesis of IFN tau action to regulate OTR gene expression during maternal recognition of pregnancy in sheep. The conceptus produces IFN tau between Days 10 and 21 of early pregnancy with peak production on Days 14 to 16. IFN tau binds to Type I IFN receptors (IFNAR) on the endometrial lumenal (LE) and superficial glandular epithelia (sGE) and, through an unknown signal transduction pathway that involves IFN regulatory factor two (IRF-2), inhibits transcription of the ER gene. These antiestrogenic actions of IFN tau prevent estrogen-induced increases in ER, PR and OTR synthesis and, hence, production of luteolytic pulses of PGF. Legend: ER, estrogen receptor alpha; IFNAR, Type I IFN receptor; OTR, oxytocin receptor; PGF, prostaglandin F2 alpha; PR, progesterone receptor. Adapted from (Spencer *et al.* 1996).

estrogen and progesterone in preparation of the mouse uterus for implantation and decidualization during early pregnancy (24).

2.2. Effects of pregnancy and IFN tau on PR, ER and OTR in Sheep

During maternal recognition of pregnancy, the conceptus trophectoderm secretes IFN tau between Days 10 and 21 with maximal production on Days 14 to 16 (25). IFN tau appears to be the major factor produced by the conceptus that prevents development of the endometrial luteolytic mechanism (see 1). In order to abrogate development of the luteolytic mechanism, IFN tau from the sheep conceptus could stabilize the PR or suppress ER and OTR gene expression (figure 2). Available evidence indicates the PR expression in the endometrial epithelia is not stabilized or increased during pregnancy (4). Furthermore, IFN tau does not stabilize or increase PR gene expression in uterine epithelia (15). Indeed, expression of PR mRNA and protein is not different in pregnant as compared to cyclic ewes on Days 11 to 15. Rather, IFN tau acts in a paracrine fashion on LE and sGE to suppress transcription of ER and OTR genes (26,27), thereby abrogating development of the endometrial luteolytic mechanism (4,17,28). The cyclic increase in ER and OTR gene expression detected in the LE and GE on Days 11 to 17 post-estrus does not occur in pregnant ewes. Therefore, IFN tau inhibits increases in OTR expression that prevent endometrial production of luteolytic pulses of PGF, but does not inhibit basal production of PGF which is actually higher in pregnant than cyclic ewes (see 1,2,3). Moreover, the antiestrogenic actions of IFN tau prevent increases in ER and thus PR expression. This facet of IFN tau action may be critical given that PR disappear prior to implantation in the endometrial epithelia of every species studied.

3. PROGESTERONE, ENDOGENOUS BETA RETROVIRUSES, AND THE CONCEPTUS

3.1. Endogenous retroviruses

A distinctive feature of retroviruses is their presence as inherited elements in the germline of most eukaryotes. These endogenous retroviruses (ERVs) are transmitted through the germline as stable Mendelian genes that exhibit structural and sequence similarities to infectious exogenous retroviruses (29). The ERVs appear to derive from integration events during evolution of ancient exogenous retroviruses (e.g. transmitted horizontally) into the germline of host animal species, including the human germline. Generally, endogenous proviruses are transcriptionally silent and are often defective due to deletions or point mutations that render them incapable to form infectious virus. However, several ERVs maintain some intact open reading frames with expression associated with either beneficial or detrimental effects to the host (30,31). Specific expression of some ERVs in placentae led to various hypotheses that these elements play a role in mammalian reproduction (see 30).

3.1.1. Jaagsiekte sheep retrovirus (JSRV)

The ovine genome contains 15 to 20 copies of endogenous beta retroviruses that are highly related to two oncogenic exogenous beta retroviruses, JSRV and enzootic nasal tumor virus (ENTV) (31). Expression of endogenous JSRVs (enJSRVs) in sheep is limited to epithelia of oviduct, uterus, cervix and vagina (31-33). In the uterus, enJSRV RNAs are among the most abundant RNA population in the entire endometrium and increase 15-fold in expression between Days 1 and 13 of the estrous cycle or early pregnancy (32). This supports the hypothesis that ancestors of the modern JSRV and ENTV did not have a tropism for the respiratory system, but for the genital tract, and may have been transmitted from sheep to sheep during mating. The enJSRVs are highly expressed in epithelia of the uterus, while the exogenous pathogenic viruses, JSRV and ENTV, have strict tropism for secretory cells of the respiratory tract (33). Expression of enJSRV RNAs is restricted to the endometrial LE and GE of the uterus and is particularly abundant, which suggests physiological functions in regulation of conceptus development, pregnancy recognition signaling by trophectoderm, and conceptus-endometrial interactions during placentation (32,33).

3.1.2. Progesterone regulation of enJSRV expression

Progesterone, acting via PR, increases transcription of enJSRV genes *in vivo* and transcriptional

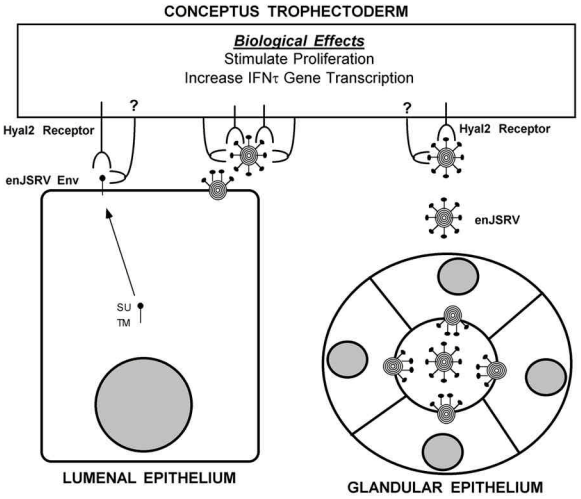

Figure 3. Schematic illustrating current working hypothesis on effects of endogenous beta retroviruses expressed in the ovine uterine endometrial epthelia on trophectoderm proliferation and production of IFN tau. One or more of the 15-20 enJSRV loci present in the ovine genome are transcriptionally active in the endometrial LE and GE. It is proposed that the enJSRVs *env* gene is inserted into the apical surface of the LE or assembled into viral particles that egress from the epithelia into the uterine lumen. The receptor for the enJSRV envelope protein is hyaluronidase 2 (Hyal2), a GPI-anchored cell surface protein, that may be expressed on the mononuclear trophectoderm cells of the elongating conceptus. Interaction of the enJSRV envelope with the Hyal2 receptor allows for viral particle entry into trophectoderm and/or initiates an unknown signaling pathway. The biological effects of this ligand-receptor interaction on the trophectoderm are stimulation of cell proliferation and production of IFN tau. Legend: enJSRV, endogenous Jaagsiekte sheep retrovirus; Hyal2 receptor, hyaluronidase 2; SU, superficial portion of envelope protein; TM, transmembrane portion of envelop protein.

activity of several enJSRV long terminal repeats (LTRs) (33). Further, JSRV capsid and envelope proteins are expressed by uterine LE and GE and detected in binucleate cells of conceptus trophectoderm that forms syncytia with uterine LE. Steady-state levels of enJSRV RNAs in LE and GE increase rapidly between Days 1 and 13 in cyclic and pregnant ewes and then decreases to low levels by Day 15 in cyclic ewes and by Day 19 in pregnant ewes. Increases in expression of enJSRV genes in uterine epithelia are highly correlated with changes in circulating levels of progesterone in peripheral blood and, most importantly, limited to the period when PR are expressed in uterine epithelia. Therefore, one or more enJSRV LTR, which contains the retroviral promoter and enhancers, are directly regulated by progesterone.

3.1.3. Coordinate expression of enJSRV and IFN tau

Expression of enJSRVs in uterine epithelia of ewes between Days 11 to 19 of pregnancy is coordinate with IFN tau production by mononuclear cells of trophectoderm (34). Perhaps enJSRV transforms conceptus trophectoderm to induce expression of proto-oncogenes such as *c-fos*, *c-jun* and *ets* that act via response elements to stimulate expression of IFN tau genes (35). Thus, pregnancy recognition signaling in ruminants may result from a primitive viral-antiviral strategy to induce production of IFN tau by trophectoderm to silence transcription of ER and OTR genes in uterine LE and sGE.

3.1.4. Effects of enJSRV on trophectoderm

In sheep, implantation is preceded by elongation of the conceptus from a tubular to filamentous forms between Days 12 and 15, an event that involves trophoblast cell rearrangement and proliferation (36,37). Conceptus elongation is temporally associated with production of IFN tau and pregnancy recognition. The expression of IFN tau genes is unusual, because they are only transcribed in the mononuclear trophectoderm cells (34) and sustained over several days rather than limited to a few hours (38). Given the proliferation of the trophectoderm during the peri-implantation period, the enJSRVs envelope protein may stimulate cell division analogous to transforming properties induced by the exogenous JSRV envelope protein (39,40). Three of the 15-20 enJSRV loci have been cloned (31), but lack the putative phosphatidylinositol 3-kinase (PI-3K) docking site in the transmembrane region necessary for transformation *in vitro* (41). Although the *gag* gene in the three cloned enJSRVs is defective, both JSRV envelope and capsid proteins are present in endometrial epithelia and trophectoderm binucleate cells (31).

The current working hypothesis is that one or more of the 15-20 transcriptionally active enJSRV loci expressed in the uterine endometrial LE and GE will encode viral particles that egress from the apical surface into the uterine lumen (figure 3). Alternatively, the enJSRVs envelope proteins may be inserted into the cell membrane of the LE and GE via the transmembrane domain (TM) with the superficial (SU) domain exposed on the apical surface. The JSRV envelope receptor is hyaluronidase 2 (Hyal2), a glycosylphosphatidyl inositol (GPI)-anchored protein (40). This receptor protein is likely to be expressed on the mononuclear trophectoderm cells and bind enJSRVs envelope proteins present on the surface of viral particles in the uterine lumen or perhaps on the LE. The resultant biological responses are proposed to include stimulation of trophoblast cell proliferation and increased production of IFN tau.

In trophectoderm binucleate cell biology, expression of enJSRVs in the endometrial epithelia may play a role in placental morphogenesis. Expression of enJSRV RNAs also occurs in binucleate cells of ovine trophectoderm that, during synepitheliochorial placentation, fuse with LE and produce PL from Day 16 of pregnancy to term (42). Binucleate trophectoderm cells fuse with the endometrial LE to form a syncytium and only binucleate cells display invasive properties. Indeed, retroviral particles are present in the placenta of many animal species including ruminants (43). Expression of the enJSRV gene in developing sheep placentae is similar to that for the human endogenous retrovirus, HERV-W (44) that is expressed in syncytiotrophoblast of the human placenta

formed by fusion of trophoblast and uterine epithelium. HERV-W envelope protein, as for many retroviral envelope proteins, induces formation of syncytia when expressed *in vitro*, thereby advancing the hypothesis that HERV-W is involved in human placental morphogenesis (45,46). Collectively, these observations support the theory that an ancient retroviral infection had profound consequences for mammalian evolution (47). The presence of enJSRV RNAs, as well as expression of both envelope and capsid proteins, suggests involvement of this endogenous betaretrovirus in synepitheliochorial placentation in sheep.

4. PROGESTAMEDINS MEDIATE EFFECTS OF PROGESTERONE

Abrogation of the luteolytic mechanism in sheep requires both IFN tau and progesterone (see 1,2,3). The actions of progesterone are mediated by the PR, but PR are not detectable in uterine LE and GE in sheep after Days 11 and 13 of pregnancy, respectively (4). However, PR is expressed in uterine endometrial stromal cells and myometrial smooth muscle cells throughout pregnancy. Thus, stromal cells must respond to progesterone and produce factors that act in a paracrine manner on uterine epithelial cells to regulate functions essential for implantation and placentation. This paradigm of loss of PR in uterine epithelia as a prerequisite for implantation has been demonstrated in sheep (4), cattle (5), pig (6), western spotted skunk (7), rhesus monkey (8), women (9), and mice (24). Therefore, implantation may be prevented if uterine LE and superficial GE express PR. However, regulation of LE and GE functions must be directed by specific factors produced by PR-positive stromal cells (see 48-50).

Progesterone is essential for the establishment and maintenance of pregnancy in ewes (see 1). The requirement for progesterone may be related to production of "progestamedins" which are involved in epithelial-mesenchymal interactions and regulation of uterine function (48). In the primate uterus, fibroblast growth factor-7 (FGF-7), also known as keratinocyte growth factor (KGF), is produced by stromal cells and appears to be a mediator of progesterone action (progestamedin) on uterine epithelia (51). This mechanism requires that progesterone act on stromal cells that express PR throughout pregnancy to stimulate production of growth factors or progestomedins that act on PR-negative epithelial cells that express receptors for progestamedins to maintain a "progestational state."

4.1. Stromal cell-derived growth factors

Three growth factors are known to be secreted by stromal cells and to act via receptors that are unique to epithelial cells. These are FGF-7 or KGF, FGF-10, and HGF. Stromal cells of the primate uterus express FGF-7 in response to progesterone, but its endocrine regulation in myometrium, tunica muscularis of arteries and placenta is not known (52). FGF-7 and FGF-10 both act via FGF receptor two IIIb (FGFR2IIIb or KGFR), whereas the receptor for HGF is encoded by the proto-oncogene *c-met* (HGF receptor). Both FGFR2IIIb and *c-met* are uniquely expressed in epithelial cells (see 52). HGF is expressed by fibroblasts and smooth muscle cells of the uterus, placenta and ovaries in rodents, humans, sheep, and horse (53). Both FGF-7 and HGF act on epithelial cells to stimulate proliferation, migration and differentiation. Although FGF-7 acts as a progestamedin, endocrine regulation of HGF expression is not clear. The primate uterus and mouse ovary express HGF in response to estrogen, but effects of progesterone and androgens on HGF expression in any tissue have not been reported. FGF-10 is also a stromal derived growth factor with similar activities to FGF-7 and has been linked directly to development of lung, brain, and limbs, as they fail to develop in mice lacking FGF-10 (see 54).

4.1.1. Fibroblast growth factor 10 (FGF-10)

In adult ewes, levels of FGF-10 mRNA are relatively high in uterine stromal cells during the luteal phase of the estrous cycle and during the peri-implantation period of early pregnancy when there are high levels of circulating progesterone and an absence of PR expression in uterine LE and GE (55). FGF-10 is a candidate progestamedin, but its endocrine regulation in the ovine uterus remains to be elucidated. FGF-10 is also expressed by chorioallantoic mesenchyme and FGFR2IIIb is expressed on adjacent trophectoderm. These results suggest that FGF-10 mediates placental mesenchymal-trophectodermal interactions to stimulate proliferation and differentiation of the placenta.

4.1.2. Fibroblast growth factor 7 (FGF-7)

In sheep, FGF-7 expression is in media intima of uterine blood vessels (55), which is consistent with its expression in spiral arteries of the primate endometrium (50). However, FGF-7 expression by stromal cells proximal to LE and GE is not detected in ewes. In the ovine uterus, cells that expressed FGF-7 are a stromal cell subtype that is different from those that express FGF-10. Further, expression of FGF-7 in the ovine uterus is very restricted whereas FGF-10 is expressed throughout the stromal compartment proximal to endometrial LE and GE. The nonoverlapping spatial patterns of expression for FGF-10 and FGF-7 in the sheep uterus suggest independent roles in uterine functions and conceptus development.

4.1.3. Hepatocyte growth factor (HGF)

HGF and its receptor *c-met* are expressed in the ovine uterus during neonatal uterine development and in the adult during the estrous cycle and pregnancy (53,56). HGF is expressed by stromal cells of the endometrium, whereas *c-met* mRNA is localized exclusively to LE and GE. HGF is also expressed by chorioallantoic mesenchyme, and *c-met* is expressed by trophectoderm. HGF may stimulate epithelial morphogenesis and differentiated function required for establishment and maintenance of pregnancy, conceptus implantation and placentation (52). HGF regulates human endometrial epithelial cell proliferation and motility (57) and mediates estrogen actions (i.e. estromedin) in the primate uterus (50). HGF expression in endometrium of cyclic ewes is highest on Days 1 and 5, decreases from Days 7 to 13, and then increases on Day 15. In pregnant ewes, HGF expression decreases between Days 11 and 13, increases from Day 13 to Days 15 and 17, and then decreases by Day 19. HGF expression is not affected by pregnancy status in ewes.

Expression of *c-met* is low between Days 1 and 7 and increases to maximal levels on Day 13 of the estrous cycle in ewes. In pregnant ewes, c-met expression increases between Days 11 and 15, remains high through Day17, and then decreases by Day 19. Expression of *c-met* mRNA was not affected by pregnancy status.

The hormonal regulation of expression of HGF and *c-met* in ovine endometrium is unknown. Ovine endometrial HGF expression increases when PR are abundant in stromal cells, but absent in LE and GE. Similarly, *c-met* expression increases in ovine endometrial epithelia when circulating levels of progesterone increase and epithelial cell PR decrease, implicating a role for progesterone in *c-met* mRNA regulation perhaps through progesterone-induced down regulation of PR. Inflammatory cytokines such as interleukin one alpha (IL-1), IL-6 and tumor necrosis factor alpha (TNF) may also affect expression of both HGF and *c-met* (58). Therefore, expression of HGF and *c-met* may be coordinated by the actions of ovarian steroids and cytokines through a complex network. In mice, HGF is required for chorioallantoic mesenchymal-trophoblastic interactions resulting in placental organogenesis (59). In sheep, *c-met* expression in trophectoderm and HGF expression in allantoic mesenchyme suggests similar roles for HGF in placental development and embryogenesis.

4.2. *Hoxa-10* and Indian Hedgehog

Recent studies in the mouse uterus have discovered that *Hoxa-10* and Indian Hedgehog (IHH) are progesterone-regulated genes that regulate stromal cell function (60,61). Hox genes are developmentally regulated transcription factors belonging to a multigene family (62). *Hoxa-10* is one *AbdominalB*-like homebox gene that is located in the Hoxa cluster and expressed in the developing genitourinary tract during mouse embryogenesis (63). *Hoxa-10* is strongly expressed in the stroma and decidua of the pregnant mouse uterus, and null mice exhibit female infertility that is due to defects in decidualization (64,65). Lim *et al.* (60) provided evidence that uterine stromal responsiveness to progesterone with respect to both PG signaling and cell proliferation is defective in *Hoxa-10* null mice. IHH, one of the family of mammalian hedgehog proteins (66), is expressed at high levels in the LE and GE of the mouse uterus on Day 3, and is downregulated in the LE but not GE on Day 4 (67). These results indicated a role for IHH as a paracrine mediator of stromal cell proliferation. Subsequently, Matsumoto *et al.* (61) provided evidence that IHH made by the epithelium functions as a paracrine growth factor for stromal cells during the early stages of mouse pregnancy. Collectively, available results indicate that "progestamedins" may originate from either epithelium or stroma and regulate epithelial-mesenchymal interactions by autocrine and/or paracrine effects.

5. INTERFERON TAU SIGNALING FOR PREGNANCY RECOGNITION REQUIRES PROGESTERONE

5.1. IFN tau signal transduction pathway

IFN tau is expressed between Days 10 and 21 of gestation and acts differentially on the endometrial LE, GE and stroma to regulate expression of a number of genes (28,68). The actions of IFN tau to signal pregnancy recognition and induce or increase expression of a number of IFN stimulated genes (ISGs) is dependent on the effects of progesterone (69,70).

Effects of IFN tau are mediated through a signal transduction system that appears to be similar to that of IFN alpha/beta?and Type I IFN receptors (IFNAR) (71-74). As illustrated in figure 4, IFN tau binds to Type I IFNARs and activates latent tyrosine kinases, janus kinase one (JAK1) and Tyk2, which phosphorylate the tyrosine residues of signal transducer and activator of transcription one (STAT1) and STAT2. These two phosphoproteins then bind a third DNA-binding protein, ISG factor 3 gamma or p48 (ISGF3 gamma), and the multimeric protein complex then translocates to the nucleus. This ISGF3 transcription factor complex binds to an IFN stimulated response element (ISRE) present in the promoter region of IFN regulatory factor one (IRF-1) and increases the rate of gene transcription. IRF-1 is a positive-acting transcription factor that binds to an IRF element (IRF-E) and increases gene transcription (75). Interestingly, IRF-E are often contained within the larger ISRE. Although the IRF-1 protein can bind to an ISRE containing an IRF-E and activate transcription, the ISGF3 transcription factor complex can not bind to an IRF-E alone. Transcription of the IRF-2 gene is increased by IRF-1 binding to IRF-Es present in the IRF-2 gene promoter region. IRF-2 is a negative-acting transcription factor that can displace IRF-1 and silence genes such as ER alpha and OTR genes to prevent luteolysis (see 27,28).

5.2. Interferon stimulated genes (ISGs)

Type I IFNs exert their biological effects by inducing expression of 30 or more ISGs that encode for proteins with antiviral, antiproliferative, immunomodulatory and antiluteolytic properties, including a growing family of IRFs (see 68,75). IFN tau, in addition to suppressing or silencing transcription of ER and OTR genes, induces or increases expression of a number of ISGs in the ruminant endometrium. These ISGs include STATs 1 and 2 (28), beta2-microglobulin (76), IRF-1 (28,77), ISG17 (78), Mx protein (79), and 2',5' oligoadenylate synthetase (OAS) (80,81). In the endometrium of early pregnant ewes, as well as cyclic ewes receiving intrauterine injections of recombinant ovine IFN tau, ISG17 and OAS genes are increased only in the endometrial stroma and middle to deep GE (see 70,81).

As illustrated in figure 4, available results from studies to determine effects of the estrous cycle, pregnancy and IFN tau on expression of STAT1, STAT2, IRF-9, IRF-1 and IRF-2 genes in the ovine endometrium indicate that: (1) in cyclic ewes, STAT1, STAT2, IRF-1 and IRF-9 expression is detectable at low levels in stroma and GE; (2) expression of ISGs is induced or increased only in the stroma and GE in pregnant ewes; and (3) expression of IRF-2 is restricted to LE and sGE of both cyclic and pregnant ewes to silence expression of selected genes, such as ER and OTR, and ISGs, including STATs 1 and 2, IRF-9 and most ISGs studied to date (see 28), including MHC

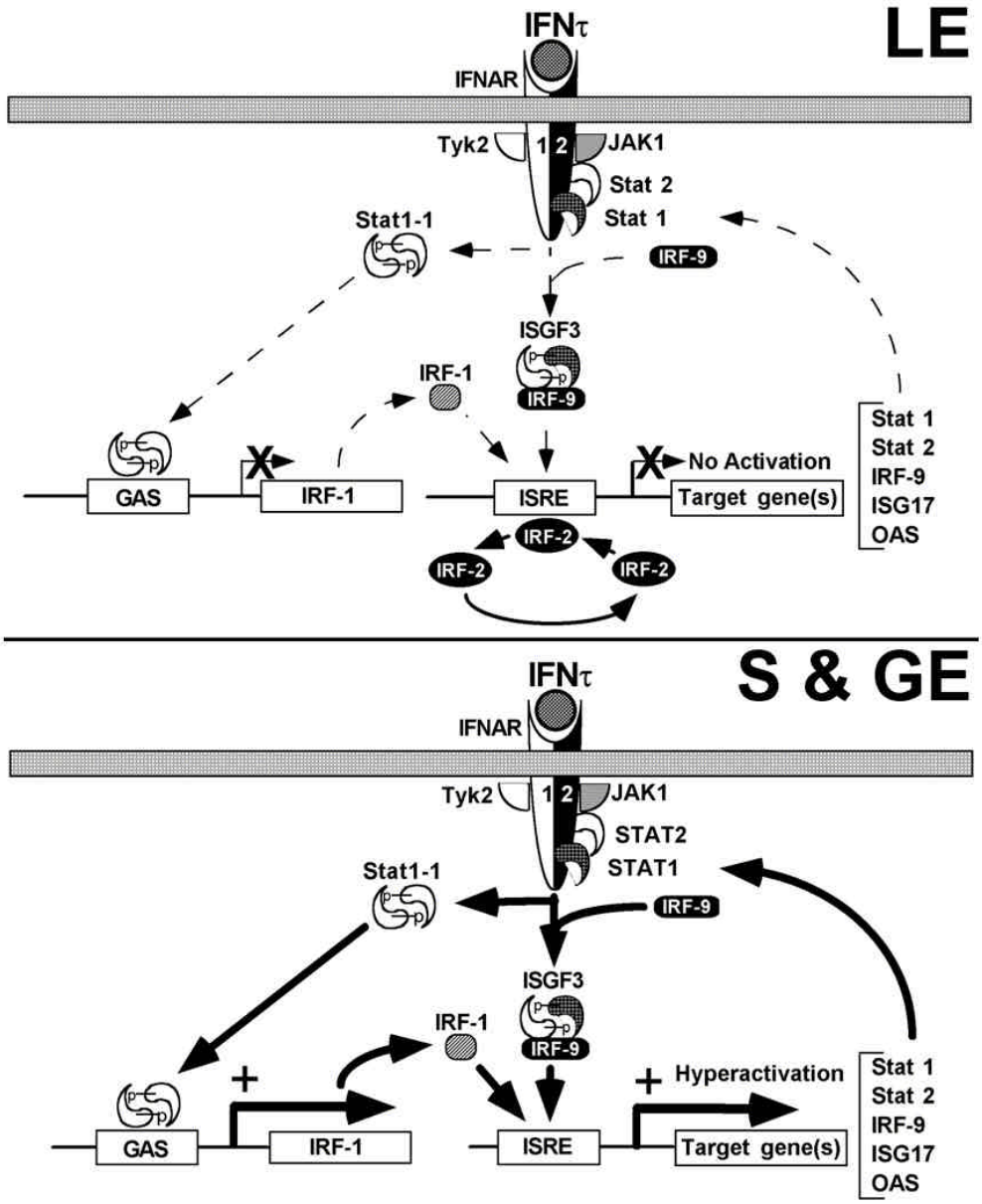

Figure 4. Schematic illustrating current working hypothesis on IFN tau signalling in endometrial epithelia and stroma of the ovine uterus. IFN tau, produced in large amounts by the developing conceptus, binds to the Type I IFN receptor (IFNAR) present on cells of the ovine endometrium. In cells of the stroma and middle to deep GE (see bottom panel), IFNτ-mediated association of the IFNAR subunits facilitates the cross-phosphorylation and activation of two Janus kinases, Tyk2 and JAK1, which in turn phosphorylate the receptor and creates a docking site for STAT 2. STAT 2 is then phophorylated, thus creating a docking site for STAT 1 which is then phosphorylated. STAT 1 and STAT 2 are then released from the receptor and can form two transcription factor complexes. ISGF3, formed by assocation of the STAT1-2 heterodimer with IRF-9 in the cytoplasm, translocates to the nucleus, and transactivates genes containing an ISRE(s), such as STAT 1, STAT 2, IRF-9, ISG17 and OAS. GAF is formed by binding of STAT 1 homodimers, which translocates to the nucleus and transactivate genes containing a GAS element(s), such as IRF-1. IRF-1 can also bind and transactivate ISRE-containing genes. The simultaneous induction of STAT2 and IRF-9 gene expression by IFN tau appears to shift transcription factor formation from GAF towards predominantly ISGF3. Therefore, IFN tau activation of the JAK-STAT signal transduction pathway allows for constant formation of ISGF3 and GAF transcription factor complexes and hyperactivation of ISG expression. In the cells of the LE and sGE, IFN tau is prevented from activating ISGs in the LE and sGE by IRF-2 (see upper panel). IRF-2, a potent and stable repressor present in the nucleus, increases during early pregnancy in LE and sGE. The continual presence of IRF-2 inhibits ISRE-containing target genes through direct ISRE binding and coactivator repulsion. Adapted from (Choi *et al.* 2001).

Class I and beta2-microglobulin (Choi Y, T.E. Spencer & F.W. Bazer, unpublished results).

5.2.1. 2',5' oligoadenylate synthase (OAS)

IFN tau induces or increases expression of a number of ISGs in the ruminant endometrium as noted previously in this review. OAS is an ISG that activates a constitutively expressed latent endonuclease, ribonuclease L that rapidly cleaves viral and cellular RNA, thereby blocking viral replication and initiating apoptosis in some cell types (82). However, OAS can also affect cell growth, differentiation and apoptosis. In cyclic ewes, low levels of OAS protein are detected only in endometrial stroma and GE, whereas OAS expression increases markedly only in stroma and GE in pregnant ewes. The absence of these ISGs in LE appears to result from the constitutive expression of IRF-2, a well-characterized transcriptional repressor of ISGs, in the LE and sGE (see 28). Intrauterine injections of IFN tau in cyclic ewes from Days 11 to 15 elicited an approximate five-fold increase in endometrial OAS on Day 16, but this effect of IFN tau was negated by a PR antagonist (81).

Interestingly, the 42-kDa form of OAS interacts with the intracellular domain of the long form of the human prolactin receptor (PRL-R) and inhibits STAT1-mediated signaling by PRL to the IRF-1 promoter (83). OAS interacts with the long form of the human PRL-R and preferentially modulates the signal transduction pathway towards activation of genes associated with differentiated function rather than proliferation. Given that ovine PL signals via either a homodimer of the ovine PRL-R or a heterodimer of ovine PRL-R and ovine GH receptor (GH-R) (84), induction of OAS expression by IFN tau during pregnancy in the ewe may influence effects of PL on the endometrial GE to enhance proliferation and differentiated functions such as secretion of UTMP and OPN (20,81). An attractive hypothesis is that the IFN tau-regulated OAS proteins play a role in the hormonal servomechanism that regulates endometrial GE secretory gene expression during pregnancy. Ovine PL can homodimerize the ovine PRL-R and heterodimerize the ovine PRL-R and ovine GH-R (84). Ovine endometrial GE expresses both PRL-R and GH-R during early pregnancy and exposure of the progesterone stimulated endometrium to IFN tau is required for ovine PL and ovine GH to stimulate production of genes encoding UTMP and OPN, which are expressed exclusively by endometrial GE (21). The mononuclear cells of the ovine conceptus trophectoderm express IFN tau as early as Day 10, but ovine PL is not expressed until Day 16 when differentiation of binucleate trophectoderm cells occurs (42). Expression of the UTMP gene, the major secretory product of the endometrial GE during pregnancy, is not detected in endometrial GE until Day 17 of pregnancy (21). Similarly, maximal expression of OPN in GE is not until after Day 19 of early pregnancy (85). The correlated ontogeny of placental IFN tau and PL production and onset of expression of OAS by GE followed by UTMP and OPN expression lends support to the concept that OAS modifies the signal transduction pathway activated by PL and perhaps GH within the endometrial GE, and that unknown effects of progesterone are permissive to these mechanisms (20). The unknown permissive effects of progesterone are likely mediated via PR-positive stromal cells that produce progesterone-induced growth factors that act as progestamedins on uterine epithelial cells.

5.2.2. ISG17 or ubiquitin cross-reactive protein (UCRP)

Another IFN tau-induced ISG is ISG17 or UCRP (see 68). Ovine ISG17 shares 87 % identity with bovine ISG17, 64 % identity with huISG15, and 31 % identity with a tandem ubiquitin repeat that conserves the Leu-Arg-Gly-Gly (LRGG) C-terminal sequence of ubiquitin that ligates to and directs degradation of cytosolic proteins *in vivo*. In ewes, both IFN tau and progesterone are required to induce expression of ISG17 in endometrial stroma and GE (70); however, PR expression is limited to stromal cells. The pathway by which progesterone regulates IFN tau induction of UCRP mRNA in the ovine endometrium is unknown; however, UCRP expression in LE and GE likely requires both a progesterone-induced progestamedin produced by PR-positive stromal cells, and IFN tau.

5.3. Cell signaling cross-talk between progestamedins and interferon tau

Progesterone actions in target tissues are poorly understood, and their elucidation complicated by possible direct and indirect effects of progesterone on endometrial cells. Direct interaction of PR with the UCRP gene promotor is unlikely, because the promoter/enhancer region of the bovine gene does not have a progesterone response element (87). As expected, the promoters for huISG15 and boISG17 contain two and five ISRE, respectively (87). Perhaps IFN tau and progesterone regulate expression of ISG17 in the ovine uterus via cross-talk between intracellular signaling pathways of the IFN tau and progestamedins from PR-positive stromal cells. IFN tau binds Type I IFNAR and stimulates JAK1 and Tyk2 which leads to activation of signal transducers and activators of transcription (STATs 1, 2 and 3). STATs 1 and 2 bind to ISGF3 gamma to form ISGF3 which translocates to the nucleus and binds an enhancer element present in ISGs, known as the ISRE, to increase transcription of ISGs (see 71). In contrast, signal transduction by tyrosine growth factor receptors (e.g., *c-met* and FGFR2IIIb) involves recruitment of STAT3 to the receptor and stimulation of phosphatidylinositol 3-kinase (PI 3-K), followed by protein kinse C (PKC) activation (88). Components of these separate signal transduction pathways may be coupled. Convergence between the JAK/STAT and PKC pathways may involve STAT3 since stimulation of the Type I IFN receptor leads to activation of STAT3 (89) and HGF stimulates recruitment of STAT3 to the *c-me*t receptor (90). Notably, ligand dependent tyrosine phosphorylation of the IFNAR1 subunit of the Type I IFN receptor complex results in recruitment and subsequent tyrosine phosphorylation of STAT3 (91). However, IFN tau alone does not stimulate long-term activation of STAT3 (72).

6. PROGESTERONE AND CONCEPTUS IMPLANTATION

6.1. Extracellular matrix proteins (ECM) and integrins

Establishment of pregnancy in mammals requires coordinated conceptus-maternal interactions involving

numerous hormones, growth factors and cytokines acting via specific receptors in the uterus. Uterine secretions play an important role in establishing synchrony between development of the conceptus and uterine receptivity, as well as in conceptus remodeling, adhesion, implantation and placentation. In sheep, integrins play a dominant role in interactions between ECM and ECM receptors to transduce cellular signals in uterine epithelial cells and conceptus trophectoderm (see 92). The endometrium exhibits both constitutive and cycle-dependent expression of integrins and appears to be the only tissue known to exhibit hormone-dependent integrin expression. Three integrins are considered markers of uterine receptivity for implantation in humans, which occurs when the uterus is under the influence of progesterone. The timing of alpha v-beta 3 expression correlates with embryo attachment and disappearance of the alpha 4 integrin subunit. The presence of both alpha v-beta 3 and alpha 5-beta 5 on the apical surface of uterine LE suggests a role for these integrins in trophectoderm-LE interactions during implantation.

6.2. Mucin glycoprotein one (MUC-1)

In both humans and rodents, the expression pattern of the glycoprotein MUC-1 on uterine LE may control accessibility of integrin receptors to their ligands and provide a barrier to invasiveness by sterically blocking cell-cell and cell-ECM adhesion and access of trophectoderm to uterine LE (see 92). The implantation adhesion cascade in rodents and sheep is initiated following down-regulation of MUC-1, which is coincidental with loss of PR from uterine epithelia (93). This pattern of MUC-1 expression contrasts with that in rabbits and humans in which there is an overall increase in MUC-1 expression during the receptive phase under the influence of progesterone; however, MUC-1 is locally reduced at implantation sites, perhaps due to paracrine signals from blastocysts that cause down-regulation of MUC-1 (see 94).

6.3. Cell adhesion and implantation

In contrast to rodents and humans, implantation in domestic animals follows an extended pre-attachment (or pre-receptive) period of 8-15 days (see 37). The pre-attachment period of pigs and sheep is characterized by migration and spacing of embryos within the uterus and then extensive conceptus remodeling as spherical blastocysts undergo elongation to filamentous conceptuses with a centrally positioned embryonic disc between Days 11 and 16 of pregnancy. This prolonged period of apposition and attachment in domestic animals offers a unique opportunity to investigate the adhesion and signal transduction events associated with initial phases of implantation without the process being obscured by rapid conceptus invasion and uterine stromal decidualization as occurs in humans and rodents (see 92).

Ruminant placentae are of the synepitheliochorial type formed when binucleate trophectoderm cells migrate and invade the LE in aglandular areas of the uterus (caruncles) and undergo a syncytial transformation defined as a fusion of the binucleate cells with the LE (42). There are also partial areas of LE degeneration that are later replaced by LE cells. In sheep, alpha (v, 4, 5) and beta (1, 3, 5) integrin subunit expression occurs in endometrium of both cyclic and pregnant ewes and conceptus trophectoderm. These integrin subunits are apically expressed on LE and GE and on conceptus trophectoderm and expression of these integrins is constitutive and not influenced by pregnancy or presence of the conceptus in sheep. In the ewe, receptivity to implantation does not appear to involve temporal and spatial patterns of integrin expression, but may depend on expression of ECM proteins such as OPN, which are ligands for heterodimers of these intergrins. Similarly, in species such as pig, mouse and humans interactions between specific integrins and ECM proteins frame the putative window of implantation. In pigs, progesterone increases expression of alpha 4-beta 1 and alpha 5-beta 1 during the peri-implantation period, which may be part of the "implantation window" in that species (see 92,95).

Conceptus development to the blastocyst stage does not appear to require histotroph secretions from the uterine glands, however, the importance of endometrial secretions on preattachment conceptus development and onset of pregnancy recognition signals was demonstrated in studies of uterine gland knockout (UGKO) ewes (see 96). The UGKO ewes have morphologically normal hatched blastocysts on Day 9 after mating, but conceptus development fails during the peri-implantation period in the absence of contributions from GE although these ewes have normal circulating levels of progesterone (97). Among the secretions of GE likely to be essential for conceptus development are proteins at the maternal-conceptus interface capable of serving as either ligands for integrins or as factors that affect their affinity state including OPN and GlyCAM-1. These proteins are absent from uterine flushings of UGKO ewes. However, the expected array of integrin receptors are expressed by LE in UGKO ewes (98).

7. PROGESTERONE MODULATION OF UTERINE MILK PROTEIN AND OSTEOPONTIN GENE EXPRESSION IN THE OVINE UTERUS

7.1. Uterine milk proteins (UTMP)

UTMPs are members of the serpin family of serine protease inhibitors (99) and serve as an excellent marker for endometrial secretory capacity during pregnancy in sheep (21,100). In pregnant ewes, UTMP mRNA expression is restricted to the endometrial GE in the deep stratum spongiosum (dGE), increases substantially between Days 15 and 17, and, between Days 17 to 50 of gestation, expression is markedly higher in upper than lower dGE. After Day 50, hyperplasia of the dGE was accompanied by increased UTMP mRNA expression by all dGE. These changes in UTMP mRNA expression are correlated with PL production by the trophectoderm and state of dGE differentiation during pregnancy.

7.2. Osteopontin (OPN)

OPN is an acidic phophorylated glycoprotein component of the extracellular matrix detected in epithelia and in secretions of many tissues, including the gastrointestinal tract, thyroid, kidney, breast, testes, oviduct, uterus, trophoblast and placenta (101). OPN binds

to alpha v-beta 3, alpha v-beta 1, alpha v-beta 5, and alpha 4-beta 1 integrin heterodimers via its Arg-Gly-Asp (RGD) sequence to promote cell adhesion, spreading and migration and also stimulates calcium transport and PI3-K activity (see 101). During the peri-implantation period, the endometrial glands produce histotroph that nourishes and sustains the conceptus during conceptus remodeling, adhesion, implantation and placentation (see 96). OPN increases in uterine flushings from pregnant ewes during the peri-implantation period (Days 11 to 17) when adherence and attachment of conceptuses to uterine LE occurs (85,86). Secreted OPN then binds integrin heterodimers expressed by trophectoderm and uterus to: 1) stimulate changes in morphology of conceptus extraembryonic placental membranes; and 2) induce adhesion between LE and trophectoderm essential for implantation and placentation. Although OPN mRNA increases only in GE of pregnant ewes, OPN protein is localized on the apical aspect of the endometrial LE, GE and conceptus trophectoderm.

7.3. Progesterone induction of UTMP and OPN expression in endometrial glands

Previous studies indicated that progesterone induces UTMP expression by ovine endometrium (100,102). For example, treatment of ovariectomized ewes with progesterone for 6 days induced low levels of UTMP mRNA and protein, whereas treatment with progesterone for 14 or 30 days greatly enhanced UTMP expression (103). The protracted nature of this progesterone effect is not typical of genes regulated by progesterone through PR in a classic transcriptional manner. In fact, loss of PR gene expression in GE appears to be required for progesterone induction of gene expression for secreted proteins by GE. For example, administration of estrogen with progesterone induced PR expression in endometrial GE and concomitantly ablated effects of progesterone alone to induce UTMP and OPN mRNA expression in the dGE (20). The contention that loss of epithelial PR is required for endometrial GE function during pregnancy is supported by studies of PR gene expression in endometrium from cyclic and pregnant ewes (4,10). During early pregnancy, PR expression is detectable in LE and GE on Day 11, but PR are undetectable in LE and superficial GE from Days 13 to 19, and are present only in stromal cells and myometrium after Day 25 of gestation in ewes (T.E. Spencer, unpublished results). Loss of PR expression by GE appears to be required for GE remodeling and differentiation and to prevent inhibition of these events by progesterone (23,104). Available evidence supports the concept that progesterone decreases PR expression in GE to allow pituitary PRL and then PL to increase UTMP mRNA expression (see below for discussion of servomechanism). This may explain why UTMP synthesis and secretion requires long-term progesterone therapy in ovariectomized ewes.

Progesterone induces expression of OPN in uterine GE, an effect ablated by a PR antagonist (105). Intrauterine infusion of IFN tau does not affect OPN gene expression or secretion regardless of steroid treatment (20). The GE that expresses OPN lack detectable PR, although PR is expressed in stroma. Thus, progesterone likely regulates OPN expression in GE through a complex mechanism that includes PR down-regulation along with stimulation of GE by a progesterone-induced stromal cell-derived growth factor(s) such as FGF-10, FGF-7 and/or HGF. Indeed, down-regulation of PR in GE may be integral to progesterone induction of expression of genes for proteins secreted by GE as administration of both estradiol and progesterone to ovariectomized ewes induces PR expression in endometrial GE followed by a dramatic decrease in expression of UTMP and OPN (20).

7. HORMONAL REGULATION AND FUNCTION OF ENDOMETRIAL GLANDS IN THE SHEEP UTERUS

8.1. Epigenetic effects of progesterone suppress uterine adenogenesis in neonatal ewes

All mammalian uteri contain endometrial glands that synthesize and secrete or transport a complex array of proteins and related substances termed histotroph that have essential roles during the course of pregnancy. Exposure of neonatal ewes to a progestin ablates endometrial gland differentiation and adult ewes display a uterine gland knockout (UGKO) phenotype characterized by the absence of endometrial glands (32). Experiments with UGKO ewes showed that a normal glandular endometrium is essential for peri-implantation conceptus survival and growth (96-99).

Postnatal uterine morphogenesis in sheep involves the emergence and proliferation of endometrial glands, development of endometrial folds and, to a lesser extent, growth of endometrial caruncular areas and myometrium (22). The progressive development of endometrial GE from the LE to the inner circular layer of myometrium is a coordinated event that involves bud formation and tubulogenesis, and is completed with coiling and branching morphogenesis. In UGKO ewes, endometrial glands do not penetrate the intercaruncular stroma regularly, nor is there a recognizable characteristic stratum spongiosum within the intercaruncular stroma (106). Thus, in sheep, development of GE appears to direct or permit differentiation of uterine stroma into subluminal stratum compactum and stratum spongiosum in intercaruncular areas of the endometrium.

8.2. Progesterone effects on growth factors and steroid receptors

Progestin-induced ablation of endometrial gland genesis in the neonatal ovine uterus does not appear to involve specific suppression of epithelial cell proliferation (106). However, evidence of focused proliferation of GE in tips of developing glands, documented in neonatal ovine and porcine uteri, as well as in the adult primate uterus, supports the idea that local microenvironmental conditions are important for gland proliferation (see 94).

In the developing neonatal ovine uterus, FGF-7, FGF-10, HGF and their epithelial receptors appear to be important growth factors associated with endometrial morphogenesis (56). Although FGF-7 is constitutively expressed in uteri from PND 1 to 56, FGF-10 and HGF

mRNA levels increased markedly after PND 21 when coiling and branching development of endometrial glands occurs in the neonatal ovine uterus. Further, progestin-induced inhibition of endometrial adenogenesis in the neonatal ewe altered expression patterns of these paracrine-acting growth factors and/or their receptors (106). Progesterone decreased expression of HGF and FGFR2IIIb to negate biological effects of HGF, FGF-7 and FGF-10; the stromal cell-derived growth factors.

In neonatal ewes, all uterine cell types are ER-positive on PND 1 and endometrial gland morphogenesis is accompanied by ER expression in emerging, proliferating and developing GE, as well as stroma (22). A requirement for ER in ovine uterine adenogenesis is supported by the finding that progestin-induced ablation of endometrial gland genesis in neonatal ewes involves suppression of epithelial ER expression (106). Ablation of endometrial gland genesis in neonatal ewes treated with norgestomet from birth may reflect loss or attenuation of ER-dependent signaling. Ovarian estradiol-17beta and growth factors, such as insulin-like growth factor one (IGF-I), IGF-II and epidermal growth factor (EGF), are likely involved in endometrial adenogenesis (22).

Proliferation of uterine GE and genesis of uterine glands may also involve PRL-R-dependent, estrogen-independent activation of ER (22). Prolactin can increase ER expression in rat decidual cells (107). Activation of both short and long forms of the PRL-R stimulates mitogen activated protein kinase (MAPK) signaling (108). Thus, PRL stimulation of PRL-R in developing uterine GE could activate the MAPK signaling cascade, resulting in serine phosphorylation, ligand-independent activation of ER, and up-regulation of ER, as well as IGF-I receptor gene expression. The PRL-R system could also be involved in regulation of development and proliferation of uterine GE. While critical experiments remain to be conducted, gland morphogenesis in the neonatal ovine endometrium is considered an ER-dependent phenomenon. Expression of PR in the neonatal ovine uterus allows progesterone to act as an “endocrine disrupter” through its epigenetic effects to inhibit endometrial adenogenesis.

8.3. Role of endometrial glands in uterine function

All mammalian uteri contain endometrial glands that synthesize and secrete or transport a complex array of proteins and related substances termed histotroph. Evidence from primate and subprimate species during the last century supports an unequivocal role for secretions of endometrial glands as primary regulators of conceptus survival, development, onset of pregnancy recognition signals, and implantation/placentation. In marsupials, carnivores and roe deer, changes in endometrial secretory activity are proposed to regulate delayed implantation (94,97,109). In rodents, several factors, including leukemia inhibitory factor (LIF) and calcitonin, are produced exclusively by uterine glands and are essential for establishment of uterine receptivity and embryo implantation (94). Uterine secretions are particularly important for conceptus survival and development in sheep, cattle, pigs, and horses, in which a prolonged period of pre-implantation conceptus development precedes superficial attachment and placentation.

Conceptus development to the blastocyst stage does not appear to require histotroph secretions from the uterine glands, however, the importance of endometrial secretions on preattachment conceptus development and onset of pregnancy recognition signals was demonstrated in studies of UGKO ewes (see 96-98). The UGKO ewes have morphologically normal hatched blastocysts on Day 9 after mating, but conceptus development fails during the peri-implantation period in the absence of contributions from GE, although these ewes have normal circulating levels of progesterone. Among the secretions of GE likely to be essential for conceptus development are proteins at the maternal-conceptus interface capable of serving as either ligands for integrins or as factors that affect their affinity state, including OPN and GlyCAM-1, which are absent from uterine flushings of UGKO ewes (98).

8.4. Uterine gland morphogenesis during pregnancy

After pregnancy recognition, maintenance of pregnancy requires reciprocal communication between the conceptus and endometrium during implantation and synepitheliochorial placentation (110). In sheep, superficial implantation and placentation is a lengthy process that begins on Days 15-16 and is not completed until Days 50-60 of pregnancy (36). During this period, the ovine uterus grows substantially in order to accommodate rapid conceptus development and growth in the latter half of pregnancy. In addition to placentomal development in the caruncular areas of the endometrium and changes in vascularity, the intercaruncular endometrial glands grow substantially in length (four-fold) and width (ten-fold) during pregnancy in ewes (21,110). These uterine glands synthesize, secrete or transport a variety of enzymes, growth factors, cytokines, lymphokines, hormones, transport proteins and other substances collectively termed histotroph (94,97,109,111). Available evidence strongly supports the theory that secretions from the endometrial epithelia influence conceptus development, onset of pregnancy recognition signals, and growth of the conceptus in species with an epitheliochorial type of placentation.

8.3. Servomechanism regulating uterine gland morphogenesis

The placentae of a number of species, including rodents, humans, nonhuman primates and ruminants, secrete hormones structurally related to pituitary PRL and GH which are termed PL (112-114). Ovine PL is produced by binucleate cells of the conceptus trophectoderm beginning on Day 16 of pregnancy (42), is detected in maternal serum by Day 50, and reaches peak levels between Days 120 and 130 days of gestation (113). The PRL-R transduces signals by PRL and PL (84).

Available results indicate that expression of ER, PR and OTR by endometrial epithelia is not affected by PL or GH (see 20). However, expression of UTMP by GE and GE density in stratum spongiosum were increased by both PL and GH only when ewes were treated with IFN tau between Days 11 and 21, and then either PL or GH and

progesterone daily from Days 16 to 29 after onset of estrus (see 20). Only PL stimulated expression of both UTMP and OPN in GE. Down-regulation of epithelial PR expression is requisite for progesterone induction of secretory gene expression, e.g., OPN and UTMP, by GE as the combination of progesterone and estradiol-17beta increased ER and PR in GE, which markedly suppressed expression of both OPN and UTMP. Thus, progesterone is required for expression of UTMP and OPN by GE that are PR-negative and must be regulated by progestamedins such as FGF-7, FGF-10 and/or HGF from PR-positive stromal cells. Further, abrogation of expression of UTMP and OPN by endometrial GE in the post-partum uterus ceases with onset of PR expression on post-partum Day 7 (Gray CA & T.E. Spencer, unpublished results).

In the rabbit and pig, interactions between lactogenic hormones and ovarian steroids have been proposed to constitute a "servomechanism" which regulates endometrial function (115,116). Interactions between PRL and progesterone increase endometrial proliferation and uteroglobin secretion in long-term ovariectomized rabbits by increasing the concentration of endometrial PR and uterine responsiveness to progesterone (117,118). This mechanism does not appear to be present in the ovine uterus, because neither PL nor GH effect endometrial PR or ER gene expression (20).

The servomechanism proposed to regulate endometrial gland proliferation and function during pregnancy in sheep is illustrated in figure 5. During pregnancy, the ovine endometrium is exposed sequentially to estrogen, progesterone, IFN tau, PL and GH, that may activate and maintain endometrial remodeling, secretory function, and uterine growth. IFN tau serves as the signal for maternal recognition of pregnancy in ruminants, is produced by mononuclear cells of the conceptus trophectoderm between Days 8 to 21 in sheep (maximally on Days 15 to 16), and acts in a paracrine manner on the adult endometrium. As described previously, enJSRVs expressed in the uterine LE and GE is proposed to regulate conceptus trophoblast elongation, proliferation and production of IFN tau. IFN tau maintains pregnancy by preventing development of the endometrial luteolytic mechanism that, in turn, maintains the CL and maternal progesterone production. Progesterone sequentially down-regulates PR expression in LE and GE, which is necessary for the onset of progesterone-induced secretory gene expression, as well as remodeling and differentiation of uterine glands. Secretion of PL by binucleate cells of the conceptus trophectoderm begins on Day 16 of pregnancy, with peak secretion occurring from Days 120 to 130 of gestation. PL can bind to the long form of the PRL-R as well as to a heterodimer of a PRL-R and GH-R (84), which is expressed exclusively by GE and increases throughout gestation. Growth hormone is secreted by the ovine placenta between Days 35 and 70 of gestation and binds to endometrial GH-R (119). Sequential intrauterine administration of ovine IFN tau to ewes from Days 11 to 15 post-estrus, followed by ovine PL from Days 21 to 25, increased proliferation of GE in the deep stratum spongiosum. Further, infusion of ovine GH into the uterine lumen from Days 21 to 25 increased uterine gland density in the deep stratum spongiosum, and increased the size of endometrial glands in the shallow stratum spongiosum (120). These studies indicate that a developmentally programmed sequence of events, mediated by specific paracrine-acting factors at the conceptus-endometrial interface, ultimately supports both endometrial remodeling and up-regulation of uterine secretory activity (i.e., increased expression of UTMP and OPN genes) during ovine gestation. Whether similar gestational servomechanisms regulate uterine gland development and function in other species remains to be determined. However, strategic manipulation of such mechanisms may offer therapeutic schemes designed to improve uterine capacity, conceptus survival and reproductive health.

The mechanism whereby effects of IFN tau permit GE to become responsive to PL and GH is not known. IFN tau may induce or up-regulate genes involved in signal transduction including janus kinases, STATs, IRFs or perhaps expression of IFN tau-regulated ISGs such as 40/42-kDa 2',5'-OAS (as described previously). Placental lactogen increases expression of both UTMP and OPN in GE, whereas GH only increases expression of UTMP. Although both PL and GH activate the JAK2-STAT5 and MAPK signal transduction pathways, individual genes may be regulated differentially by these placental hormones. The ability of PRL, PL and GH to elicit similar effects on the endometrial glands is not surprising given that these hormones constitute a unique hormone family based on genetic, structural, binding, receptor signal transduction and function studies. Future investigations into mechanisms regulating the process of uterine gland development and endometrial morphogenesis will provide insight into factors affecting early embryonic survival and development in humans and livestock. In particular, there is a clear need to understand convergence of interactions between cell signaling events mediated by progesterone acting via PR, progestamedin growth factors acting via their respective receptors, lactogenic hormones acting via PRL-R and GH-R and IFN tau acting through the Type I IFN receptor that regulate proliferation and differentiated functions of uterine stromal cells and GE throughout gestation.

9. CONCLUSIONS AND PERSPECTIVE

Progesterone and PR are critical components of uterine biology and the biology of pregnancy, as well as mammogenesis and lactogenesis. In the neonate, expression of PR by endometrial cells render uterine adenogenesis vulnerable to progesterone which acts as an endocrine disrupter characterized by the UGKO phenotype. In the sexually mature female, progesterone and PR effects during both the estrous cycle and pregnancy can be understood only when both temporal and spatial aspects of PR expression are considered. It seems clear that uterine stromal cells are always PR-positive and respond to PR by producing paracrine factors that regulate proliferation and/or differentiated functions of GE and LE, especially GE during pregnancy. This poses interesting questions. Why are PR negatively autoregulated in LE and GE, but

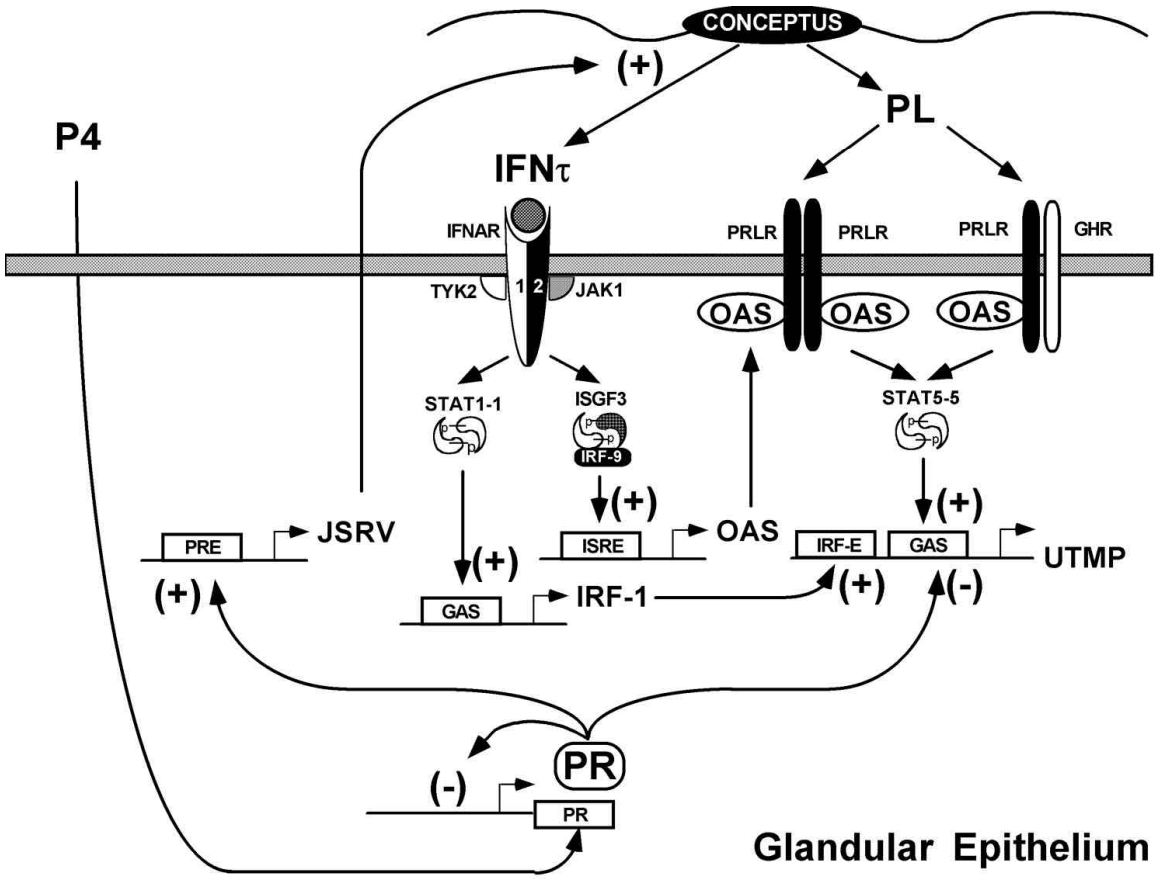

Figure 5. Schematic illustrating current working hypothesis on the hormonal and cellular mechanisms regulating IFN tau and uterine milk protein (UTMP) gene expression in the endometrial glandular epithelium. IFN tau is produced by the conceptus between Days 11 to 21-25 of pregnancy with maximal production on Days 15-16. High levels of endogenous Jaagsiekte sheep retroviruses (enJSRVs) are expressed in the PR-positive endometrial LE and GE in response to increasing progesterone and are hypothesized to stimulate trophoblast proliferation and production of IFN tau. Continuous exposure of the endometrium to progesterone for 8 to 10 days negatively autoregulates PR expression, so that LE and GE are PR-negative by Days 11 and 15, respectively. IFN tau activates the JAK-STAT pathway in the endometrial glands which stimulates formation of STAT1 homodimers (or GAF) as well as the transcription factor IFN stimulated gene factor 3 (ISGF3; heterotrimer of STAT1, STAT2 and IRF-9). STAT1 homodimers or GAF transactivate a GAS element in the IRF-1 gene. IRF-1 then binds to IRF-Es and transactivates the UTMP promoter. ISGF3 transactivates ISREs present in the 2',5' oligoadenylate synthetase (OAS) gene. The 40/46-kDa form of OAS interacts with the intracellular domain of the prolactin receptor (PRL-R) which mediates the actions of ovine PL. Specifically, OAS prevents PRLR signaling to STAT1 and promotes signaling through STAT5. Ovine PL is produced by the conceptus beginning on Days 16-17 of pregnancy which is concomitant with the formation of binucleate cells in the trophectoderm. The actions of oPL are mediated by PRLR homodimers or perhaps heterodimers of PRLR and growth hormone receptor (GHR) that stimulate formation of STAT5 homodimers. STAT5 dimers bind and transactivate the GAS element in the UTMP promoter. The induction of UTMP gene expression in the GE by IFN tau-stimulated IRF-1 is maintained by the actions of oPL through STAT5.

not stromal and myometrial cells? What is the molecular mechanism whereby progesterone down-regulates expression of the PR gene in the epithelia but not stroma of the uterine endometrium? What are the mechanisms whereby stromal cells regulate epithelial cell functions in reproductive tissues? How do cell signaling pathways activated by growth factors, interferons and lactogenic hormones converge to establish the servomechanism associated uterine functions that are critical to maintenance of pregnancy in ewes and probably in many species of mammals? Are there other endogenous retroviruses, like enJSRV, that are regulated by progesterone and PR that are expressed transiently to affect uterine biology and/or conceptus functions or mammary gland biology? We have learned much about effects of progesterone and expression of PR, but there are obviously many unresolved questions about the extent and magnitude of the effects of this key hormone of the estrous/menstrual cycle and pregnancy and its receptor. We must continue to address these and other questions if we are to enhance reproductive efficiency,

provide successful therapies to cure or ameliorate effects of hormone-dependent diseases of reproductive tract and mammary glands, and improve the health and well-being of women and children throughout the world.

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Send correspondence to: Fuller W. Bazer, PhD, 442 Kleberg Center, 2471 TAMU, Texas A&M University, College Station, TX 77843-2471, Tel: 979-845-5929, Fax: 979-862-2662, E-mail: fbazer@cvm.tamu.edu